

Amendments to the Specification:

Please replace paragraph [22] beginning at page 7, line 12, with the following:

--[22] Figure 1 depicts the plasmid construct of vector pET-11a-memapsin 2-T1 and pET-11a-memapsin 2 T2. The T7 promoter, amino acid sequence from the vector (T7 protein) (SEQ ID NO:3), and the beginning and ending of the memapsin 2 T1 and T2 construct are shown (SEQ ID NOS:32-34). Construct promemapsin 2-T1 was used in the preparation of protein for crystallization and includes residues 1v-15v which are derived from vector pET-11a. Residues 1p-48p are putative pro-peptide. Residues 1-393 correspond to the mature protease domain and C-terminal extension. The residue numbering of memapsin 2 starts at the aligned N-terminal position of pepsin (Figures 3A and 3B).--

Please replace paragraph [24] beginning at page 7, line 25, with the following:

--[24] Figures 3A and 3B are the chemical structures of memapsin 2 inhibitors, OM99-1 (SEQ ID NO:27) and OM99-2 (SEQ ID NO:35).--

Please replace paragraph [29] beginning at page 8, line 14, with the following:

--[29] Figure 8 is a schematic presentation of interaction between OM99-2 (SEQ ID NO:35) and memapsin 2 protease domain. The S₃' and S₄' subsites are not defined.--

Please replace paragraph [110] (Table 1) beginning at page 33, line 5, with the following (see attached replacement table):

Please replace paragraph [116] beginning at page 36, line 2, with the following:

--[116] Based on the results of specificity studies of memapsin 2, it was predicted that good results for positions P1 and P1' would be Leu and Ala. It was subsequently determined from the specificity data that P1' preferred small residues, such as Ala and Ser. However, the crystal structure (determined below in Example 9) indicates that this site can accommodate a lot of larger residues. It was demonstrated that P1' of memapsin 2 is the position with the most stringent specificity requirement where residues of small side chains, such as Ala, Ser, and Asp, are preferred. Ala was selected for P1' mainly because its hydrophobicity over Ser and Asp is favored for the penetration of the blood-brain barrier, a requirement for the design of a memapsin 2 inhibitor drug for treating Alzheimer's disease. Therefore, inhibitors were designed to place a transition-state analogue isostere between Leu and Ala (shown as Leu*Ala, where * represents the transition-state isostere, -CH(OH)-CH₂-) and the subsite P4, P3, P2, P2', P3' and P4' are filled with the beta-secretase site sequence of the Swedish mutant from the beta-amyloid protein. The structures of inhibitors OM99-1 and OM99-2 are shown below and in Figures 3A and 3B, respectively:

OM99-1: Val-Asn-Leu*Ala-Ala-Glu-Phe (SEQ. ID NO. 27) (SEQ ID NO:27)

OM99-2: Glu-Val-Asn-Leu*Ala-Ala-Glu-Phe (SEQ. ID NO. 28) (SEQ ID NO:35)--

The Leu*Ala dipeptide isostere was synthesized as follows:

Please replace paragraph [137] beginning at page 47, line 5, with the following:

--[137] Crystal trials were performed on purified memapsin 2 in complex with a substrate based transition-state inhibitor OM99-2 with a $K_i = 10$ nM. OM99-2 is equivalent to eight amino-acid residues (including subsites S4, S3, S2, S1 S1', S2', S3' and S4' in a sequence EVNLAAEF (SEQ ID NO:28)) with the substitution of the peptide bond between the S1 and S1' (L-A) by a transition-state isostere hydroxyethylene. Purified M2 was concentrated and mixed with a 10 fold excessive molar amount of inhibitor. The mixture was incubated at room temperature for 2-3 hours to optimize the inhibitor binding. The crystallization trial was conducted at 20°C using the hanging drop vapor diffusion procedure. A systemic search with various crystallization conditions was conducted to find the optimum crystallization condition for memapsin 2/OM99-2 inhibitor complex. For the first step, a coarse screen aimed at covering a wide range of potential conditions were carried out using the Sparse Matrix Crystallization Screen Kits purchased from Hampton Research. Protein concentration and temperature were used as additional variables. Conditions giving promising (micro) crystals were subsequently used as starting points for optimization, using fine grids of pH, precipitants concentration etc.

Please replace paragraph [142] beginning at page 49, line 3, with the following:

--[142] Molecular replacement was performed with data in the range of 15.0-3.5 Å using program AmoRe, CCP4 package[Navaza, J., Acta Crystallog. Sect. A. 50, 157(1994)]. Pepsin, a human aspartic protease with 22% sequence identity (SEQ ID NO:31), was used as the search model(PDB id lpsn). Rotation and translation search, followed by rigid body refinement, identified a top solution and positioned both molecules in the asymmetric unit. The initial solution had a correlation coefficient of 22% and an R-factor of 0.51. The refinement was carried out using the program CNS [Brunger et al., Acta Crystallogr. Sect. D, 54, 905 (1998)]. 10% of reflections were randomly selected prior to refinement for R_{free} monitoring [Bruger, A.T., X-PLOR Version 3.1: A system for X-ray Crystallography and NMR, Yale University Press,

New Haven, CT (1992)]. Molecular graphics program [Jones, T.A., et al., Improved methods for building protein models in electron density maps and location of errors in these models. *Acta Crysallogr. Sect. A* 47, 110 (1991)] was used for map display and model building. From the initial pepsin model, corresponding amino acid residues were changed to that of memapsin 2 according to sequence alignment. The side chain conformations were decided by the initial electron density map and a rotomer library. This model was refined using molecular dynamics and energy minimization function of CNS [Bruger, A.T., et al., *Acta Crystallogr. Sect. D*, 54, 905 (1998)]. The first cycle of refinement dropped the R_{working} to 41% and the R_{free} to 45%. At this stage, electron densities in the omit map clearly showed the inhibitor configuration in the active site cleft. Structural features unique to memapsin 2 in chain tracing, secondary structure, insertions, deletions and extensions (as compared to the search model) are identified and constructed in subsequent iterations of crystallographic refinement and map fitting. The inhibitor was built into the corresponding electron density.--

Please cancel the present "SEQUENCE LISTING", pages 1-17, submitted for parent application USSN 09/603,713 on June 27, 2000, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 17, at the end of the application.